

PATENT SPECIFICATION

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(54) STERILIZATION

SPECIFICATION NO 1297489

By a direction given under Section 17(1) of the Patents Act 1949 this application proceeded in the name of WAVE ENERGY SYSTEMS INC., of 600 Madison Avenue, New York, New York 10022, United States of America, a Corporation organised under the laws of the State of New York, United States of America.

THE PATENT OFFICE

R 16736/8

- The most common methods of sterilization involve either the use of steam under pressure or dry heat. However, these methods are cumbersome, tedious and time consuming, often damage the sterilized material and require expensive equipment and skilled technicians.
- Attempts have been made to develop substitutes for the above methods. The power of a chemical sterilization agent is best measured by its ability to kill sporulating bacteria, while many of the disinfectants can kill vegetative bacteria, very few are capable of killing spores, such as those of *Bacillus subtilis*, *Bacillus pumilus*, *Clostridium sporogenes* and *Clostridium tetani*.
- Recently there has been marketed an aqueous solution of glutaraldehyde buffered to an alkaline pH; generally around 7.5 to 8.0, which solution is effective in killing such spores at temperatures of around 20 to 25°C. This treatment is very time-consuming and involves generally from 3 to 10 hours to achieve complete deactivation. U.S. Patent 3,016,328 issued January 9, 1962 to Rollin E. Pepper et al and assigned to Ethicon, Inc., describes such material. It is stated in that patent that acid aqueous solutions of glutaraldehyde are not effective in killing or deactivating sporulating bacteria.
- The use of alkaline solutions of glutaraldehyde as sterilizing agents suffers from a number of defects. Firstly, aqueous solutions of glutaraldehyde are inherently acidic and to achieve the required alkaline solution, it is necessary to carefully buffer the acidic aqueous
- This clearly gives rise to problems. Thus, the container of buffer may become detached from the container of aqueous glutaraldehyde. Alternatively, the addition of buffer may be omitted. In either case this leads to the use of a solution known to be ineffective at 20° to 25°C.
- It has been found in accordance with one aspect of the present invention that aqueous acid solutions of glutaraldehyde may be used as effective sterilizing agents in killing sporulating bacteria, such as *Bacillus subtilis*, at temperatures above 45°C. Thus, it is possible to market an aqueous acid solution, which has a long shelf life, and avoid the problems associated with the separate provision of buffers which must be mixed in with the acid solution.
- Preferably the temperature of sterilization is 65° to 70°C, since at these temperatures sporulating bacteria are rapidly deactivated. Deactivation with acid solutions at those temperatures is much more rapid than alkaline solutions at 25°C.
- The concentration of the aqueous glutaraldehyde solution may vary over a wide range, but generally solutions of concentrations up to 7.5% by weight may be effectively utilized. Preferably, the concentrations vary between 1 and 2% by weight.
- As indicated above, in this aspect of the present invention, the aqueous glutaraldehyde solution is acid. The pH may be varied in the acid region by suitable buffering. Generally, the pH varies between 2 and 5. It is

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(54) STERILIZATION

- (71) We, ONTARIO RESEARCH FOUNDATION, of Sheridan Park, Ontario, Canada, a corporation organized under the laws of the Province of Ontario, Canada, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—
- This invention relates to sterilization, particularly to the sterilization of laboratory, surgical, dental and other equipment in the liquid phase.
- The most common methods of sterilization involve either the use of steam under pressure or dry heat. However, these methods are cumbersome, tedious and time consuming, often damage the sterilized material and require expensive equipment and skilled technicians.
- Attempts have been made to develop substitutes for the above methods. The power of a chemical sterilization agent is best measured by its ability to kill sporulating bacteria, while many of the disinfectants can kill vegetative bacteria, very few are capable of killing spores, such as those of *Bacillus subtilis*, *Bacillus pumilus*, *Clostridium sporogenes* and *Clostridium tetani*.
- Recently there has been marketed an aqueous solution of glutaraldehyde buffered to an alkaline pH; generally around 7.5 to 8.0, which solution is effective in killing such spores at temperatures of around 20 to 25°C. This treatment is very time-consuming and involves generally from 3 to 10 hours to achieve complete deactivation. U.S. Patent 3,016,328 issued January 9, 1962 to Rollin E. Pepper et al and assigned to Ethicon, Inc., describes such material. It is stated in that patent that acid aqueous solutions of glutaraldehyde are not effective in killing or deactivating sporulating bacteria.
- The use of alkaline solutions of glutaraldehyde as sterilizing agents suffers from a number of defects. Firstly, aqueous solutions of glutaraldehyde are inherently acidic and to achieve the required alkaline solution, it is necessary to carefully buffer the acidic aqueous solution. Generally, this has been achieved by utilizing sodium bicarbonate or other mild base.
- Secondly, while acid aqueous solutions of glutaraldehyde are stable and have long shelf-life, alkaline solutions by contrast tend to lose their sporicidal activity upon storage. This latter defect has led to the marketing of the product in the form of an aqueous acid solution of glutaraldehyde together with a separate container of solid buffer, the buffer is added to the acid solution just prior to use to render the solution alkaline.
- This clearly gives rise to problems. Thus, the container of buffer may become detached from the container of aqueous glutaraldehyde. Alternatively, the addition of buffer may be omitted. In either case this leads to the use of a solution known to be ineffective at 20° to 25°C.
- It has been found in accordance with one aspect of the present invention that aqueous acid solutions of glutaraldehyde may be used as effective sterilizing agents in killing sporulating bacteria, such as *Bacillus subtilis* at temperatures above 45°C. Thus, it is possible to market an aqueous acid solution, which has a long shelf life, and avoid the problems associated with the separate provision of buffers which must be mixed in with the acid solution.
- Preferably the temperature of sterilization is 65° to 70°C, since at these temperatures sporulating bacteria are rapidly deactivated. Deactivation with acid solutions at those temperatures is much more rapid than alkaline solutions at 25°C.
- The concentration of the aqueous glutaraldehyde solution may vary over a wide range, but generally solutions of concentrations up to 7.5% by weight may be effectively utilized. Preferably, the concentrations vary between 1 and 2% by weight.
- As indicated above, in this aspect of the present invention, the aqueous glutaraldehyde solution is acid. The pH may be varied in the acid region by suitable buffering. Generally, the pH varies between 2 and 5. It is

preferred to utilize an aqueous glutaraldehyde solution in the absence of any buffers and hence at the natural acid pH of the particular solution.

5 In certain instances, the solution may have a corrosive effect, depending on the acidity employed or the nature of the object to be sterilized. It is possible to incorporate anti-corrosion agents in the solution to counteract this effect.

10 Ultrasonic energy has been proposed for rendering the human arm and hand surgically clean in a device using bactericides, as may be seen in U.S. Patent 3,481,687 issued December 2, 1969 to Sherman S. Fishman.

15 In this patent, however, no specific mention is made regarding the destruction of spores or synergistic effects between certain chemicals and ultrasonics. No value of acoustic energy density is given in the patent and it is said that a device is to be used to achieve cavitation which does not produce harmful biological side effects and pain. This means automatically that no resistant spore can be destroyed since the density of acoustic energy for effective spores destruction has to be far above the one corresponding to pain threshold.

20 In another report (E. O. Shaner, Augmentation of Bactericidal Action of Germicides with ultrasound, Jour. Acou. Soc. Am., 36:2238, 1964) it has been shown that saponification of germicidal solutions increases the bacterial efficiency. Vegetative organisms were also said to be quickly destroyed but spores were not.

25 In other reports (A Study of the Effectiveness of the Steri-Sonic 400, Micro-biocidal Agent on Pathogenic Organisms, Parkin Biochem Corp., Highwood, Calif.) which deal with the destruction of pathogens at high temperature (95°C) through a combination of ultrasonic energy at 400 KHz and a wetting agent (Tween 40) no positive results were claimed with thermoresistant spores.

30 In accordance with a second aspect of the present invention, the sterilizing action of an acid aqueous solution of glutaraldehyde is enhanced by the use of ultrasonic sound energy.

35 It has been found that where such ultrasonic sound energy is employed in addition to the acid glutaraldehyde, then the acid glutaraldehyde solution may be utilized at lower temperatures, generally down to 15°C. Sterilizing action may be obtained at approximately room temperature (i.e. 25°C). It is preferred, however, to use an elevated temperature, particularly 55° to 65°C.

40 The frequency of the ultrasonic sound may be any convenient value, generally up to 300 KHz. Sound in the sonic frequency range also may be employed satisfactorily. The frequency of the sound energy employable in this second aspect of the invention varies between 45 8 and 300 KHz, preferably 15 to 45 KHz and

particularly 20 KHz.

The energy density of application of the sonic energy to aqueous glutaraldehyde solution may vary over a wide range, typically from 10 watts per litre to 5 watts per cubic centimetre.

50 The sonic energy also may be employed in conjunction with the afore-mentioned aqueous alkaline glutaraldehyde solutions. Such alkaline solutions, which preferably have a pH of 7.5 to 9, may be utilized over a range of temperature and such solutions may effectively be used at 25°C. It is preferred to use an elevated temperature of 55° to 65°C to enhance the speed of the sporicidal activity of the solution. At these temperatures, the sound energy enhances the sterilizing ability of the glutaraldehyde solution.

55 The combination of sonic energy with a glutaraldehyde solutions leads to the provision of readily operable sterilizing equipment for use in sterilizing surgical and dental instruments.

The invention is illustrated by the following Examples:

Example I

60 A number of acid aqueous glutaraldehyde solutions of 1% concentration were prepared having pHs 2.6, 3.7 and 4.7. The effectiveness of these solutions was tested on spores of *Bacillus subtilis* at 70°C. It was found that the spores were complete inactivated in 5 minutes.

Example II.

65 The effectiveness of an acid aqueous glutaraldehyde solution of 1% concentration and pH 3.3 was tested on *Bacillus subtilis* at various temperatures, namely 45°C, 55°C and 65°C. Significant deactivation of the spores occurred at each temperature and complete deactivation was achieved in 10 minutes at 65°C.

By way of contrast, a 2% glutaraldehyde aqueous solution at pH of 8.0 at 25°C took approximately 3 hours to deactivate the spores.

Example III.

70 An aqueous alkaline glutaraldehyde solution at 1% concentration having a pH of 8.0 was tested for effectiveness as a sterilizing agent in the presence of ultrasonic energy of 20 KHz frequency and at an energy density level higher than 10 watts/litre. The tests were carried out on *Bacillus subtilis* spores one at a temperature of 25°C and another at a temperature of 55°C. It was found that at the temperature of 25°C while some inactivation of the spores at 25°C was achieved in about 30 minutes, complete inactivation of the spores at 55°C was achieved in about 5 minutes.

Example IV.

75 An aqueous acid glutaraldehyde solution at

1% concentration having a pH of 2.2 was tested for sterilizing activity on *Bacillus subtilis* spores in the presence of ultrasonic energy of 20 KHz frequency and at an energy density level higher than 10 watts/litre at 60°C. Complete deactivation of the spores occurred in about 5 minutes. This result is in contrast to that in Example II where a similar acid solution was tested in the absence of ultrasonic energy, and it was found that a 10 minutes treatment at 65°C was necessary for deactivation of the spores. This shows the enhanced effect due to synergism between the ultrasonic energy and the chemical.

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Example V.

The aqueous glutaraldehyde solution used in Example III was tested at 25°C in the presence of ultrasonic sound of a frequency of 250 KHz at an energy density level higher than 10 watts/litre. Complete deactivation of the *Bacillus subtilis* spores was achieved in about 30 minutes.

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WHAT WE CLAIM IS.—

1. A method of sterilizing a contaminated object which comprises contacting the object with an aqueous solution of glutaraldehyde having an acid pH at a temperature above 45°C.

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2. A method as claimed in claim 1, in which the aqueous solution has a pH of 2 to 5.

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3. A method as claimed in claim 1 or 2, in which the temperature is 65° to 70°C.

4. A method as claimed in any of claims 1 to 3, in which the aqueous solution has a concentration of 1 to 2% by weight.

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5. A method as claimed in claim 1 substantially as hereinbefore described with reference to either of Examples I and II.

6. A method of sterilizing a contaminated object which comprises contacting the object with an aqueous solution of glutaraldehyde while simultaneously subjecting the solution to sound energy having a frequency of from 8 KHz to 300 KHz at a power of 10 watts/litre to 5 watts/cubic centimetre.

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7. A method as claimed in claim 6, in which the sound energy has a frequency of 15 to 45 KHz.

8. A method as claimed in claim 6 or 7, in which the sound energy has a frequency of 20 KHz.

9. A method as claimed in any of claims 6 to 8, in which the aqueous solution has a pH of 2 to 5.

10. A method as claimed in any of claims 6 to 8, in which the aqueous solution has a pH of 7.5 to 9.

11. A method as claimed in any of claims 6 to 10, in which the contact takes place at a temperature in excess of 15°C.

12. A method as claimed in claim 11, in which the temperature is 25°C.

13. A method as claimed in claim 11, in which the temperature is 55° to 65°C.

14. A method as claimed in claim 6, in which the aqueous solution has a pH of 2 to 5 and a concentration of 1 to 2% by weight, the sound energy has a frequency of 20 KHz and the contact takes place at a temperature of 55° to 65°C.

15. A method as claimed in claim 6, in which the aqueous solution has a pH of 7.5 to 9 and a concentration of 1 to 2% by weight, the sound energy has a frequency of 20 KHz, and the contact takes place at a temperature of 55° to 65°C.

16. A method as claimed in claim 6, in which the aqueous solution has a pH of 7.5 to 9 and a concentration of 1 to 2% by weight, the sound energy has a frequency of 250 KHz and the contact takes place at a temperature of 25°C.

17. A method as claimed in claim 6 substantially as hereinbefore described with reference to any of Examples III to V.

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